PHYCOREMEDIATION OF MEAT PROCESSING WASTEWATER BY USING MICROALGAE *SCENEDESMUS* SP.

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DEDICATION

Especially to my beloved family, my supervisor and friends

For giving me infinite care and blessing

Thank you for your endless support to me

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ABSTRACT

Wastewater produced from Meat Processing Wastewater (MPWW) contains high nutrient loads which can induce eutrophication and cause water quality to deteriorate as it has been claimed that this type of wastewater is difficult to treat due to different types of processed meat and factory activities. The objective of this study was to analyse the ability of phycoremediation technology in order to assess its potential use as in treating MPWW and biomass by-product production. Scenedesmus sp., a type of microalgae, was employed to investigate the effects of a number of factors namely pH, temperature, light intensity, photo period and reduction in nutrients. The factors were measured through the growth rate of biomass productivity and removal efficiency. Subsequently, the design expert method was used to predict the optimal flocculation efficiency of aluminium sulphate for biomass harvesting. It was found that Scenedesmus sp. has a higher biomass productivity in the pH range of 7 to 8, temperatures between 25°C to 40°C, light intensity at 4000 lux and a photoperiod of 12 hours light/12 hours dark. These conditions show a significant influence on biomass productivity at a concentration of 1×106 cells/mL. Furthermore, the highest removal of nutrients was up to 77% for ammonia, orthophosphate, total nitrogen and total phosphorus, followed by chemical oxygen demand at 64%. On the other hand, the optimum flocculation efficiency of 97.54% was found to be best for biomass harvesting. These discoveries are significant as they showed that phycoremediation is suitable and has the potential to be implemented in treating MPWW, specifically in tropical Malaysia.

ABSTRAK

Air sisa buangan dari kilang pemprosesan daging mengandungi tinggi kandungan nutrient dimana jika berlebihan oleh menyebabkan eutrofikasi dan kemerosotan kualiti air. Disamping itu, air sisa buangan dari pemprosesan daging sukar dirawat oleh kerana kepelbagaian jenis daging dan aktiviti kilang yang beroperasi. Objektif kajian ini adalah untuk manganalisa keupayaan teknologi phycoremediation dalam merawat air sisa pemprosesan daging disamping melihat potensi penghasilan biomas. Mikroalgal sepisis *Scenedesmus* dipilih untuk dianalisa terhadap beberapa factor iaitu pH, suhu, intensity cahaya, tempoh pengcahayaan dan kepekatan mikro-algal melalui kadar pertumbuhan produktiviti biomas serta penyingkiran nutrient. Seterusnya, aplikasi *Response Surface Method* digunakan bagi menganalisa kesesuaian dos aluminium sulfate dalam penuaian biomas. Hasil kajian mendapati bahawa kadar pertumbuan produktiviti biomas adalah tertinggi pada keadaan pH 7 hingga pH 8, suhu antara 25°C hingga 40°C, keamatan cahaya pada 4000 lux, fotoperiod pada 12/12 terang : gelap dan 1×10^6 cells/mL kepekatan awal spesis *Scenedesmus*. Disamping itu, penyingkiran nutrien tertinggi adalah sehingga 77% untuk ammonia, ortofosfat, nitrogen dan fosforus, diikuti *oleh chemical oxygen demand, COD* pada 64%. Penemuan ini penting kerana ia menunjukkan bahawa teknologi phycoremediation adalah sesuai, mempunyai potensi serta sebagai kaedah alternative untuk dilaksanakan dalam merawat air sisa pemprosesan daging, khususnya di iklim tropikal Malaysia.

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SYMBOLS AND ABBREVIATIONS

MPWW	=	Meat Processing Wastewater
BOD	=	Biological Oxygen Demand
COD	=	Chemical Oxygen Demand
TP	=	Total Phosphorus
TN	=	Total Nitrogen
TSS	=	Total Suspended Solids
H	=	Hydrogen
C	=	Carbon
μmax	=	Maximum Growth Rates
sp.	=	Species
ICP-MS	=	Inductively coupled plasma mass spectrometry
RSM	=	Response Surface Method
GC-MS	=	Gas chromatography mass spectrometry
BBM	=	Basal Bold Medium
TOC	=	Total Organic Compound
PO_4^{3-}	=	Orthophosphate
IC	=	Inert Carbon

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Wastewater is defined as effluent that is generated by residential homes, agricultural activities, commercial properties as well as industrial industries. In other words, wastewater is a word that represents water that has been contaminated with pollutants and microbes (Sriram & Seenivasan, 2012). Wastewater represents a major global matter in developed and developing countries (Dube, 2010). It is related with the discharge of untreated or partially treated wastewater into the water bodies which leads to water pollution. This is due to the high content of toxic pollutants and pathogenic contaminants in these wastes (Amaefule *et al.,* 2011). Specifically, industrial wastewater is more complicated in terms of its characteristics, especially wastewater from meat processing food industries due to chemical usage during the manufacturing process. Therefore, a proper treatment process is required to ensure that effluents produced from these facilities meet acceptable wastewater effluent standards set by the authorities (Barnes *et al.,* 1984; Aris *et al.,* 2000).

The most common food processing wastewaters found in previous studies come from breweries, vegetable oil factories and other industries (Abdalla, 2014). Wastewater produced by meat processing and food industrial activities consume significantly larger quantities of fresh water. According to Bustillo & Mehrvar, (2015) and Bustillo-Lecompte *et al.,* (2016), meat processing industries among are the industries that consume up to 24% water of freshwater, making them a major

producer of wastewater. As reported by Sroka *et al.,* (2004), meat processing facilities use roughly about 62 mm^3 of water per year. Lu *et al.,* (2015) reported that meat processing facilities produce approximately 10,000m^3 of water in the USA each day. Unlike other types of wastewater such as municipal and agriculture wastewater, there is no specific wastewater treatment for meat processing industrial wastewater. The pollutant parameters of meat processing wastewater (MPWW) are commonly characterised by biological oxygen demand (BOD) and chemical oxygen demand (COD) contents along with other recoverable nutrients such as nitrogen, phosphorus, total organic carbon and total suspended solids (TSS) (Qasim & Mane, 2013; Bustillo & Mehrvar, 2015; Bustillo-Lecompte *et al.,* 2016). For years, the inadequate treatment of MPWW has been a major problem for the government. The problem normally occurs during the disposal of the MPWW that contains large quantities of pollutants as claimed by Echiegu & Liberty, (2013).

To date, there has been many attempts done by researchers to find the best treatment system for wastewater generated by food-based industries including the two-phase aerobic treatment to treat wastewater from the biscuits industry (Ibrahim *et al.,* 2013); two-stage air floatation treatment to treat slaughter house wastewater (Al-Mutairi *et al.,* 2008); two-hybrid system applying activated sludge and ultrafiltration in the treatment of meat wastewater (Bohdziewicz & Sroka, 2006), membrane bioreactor, (MBR) (Merz *et al.,* 2007) and many more. The treatment process used usually depends on financial status, location of treatment plants, other supporting equipment, standard of living as well as quality of life (Bustillo-Lecompte *et al.,* 2016). Moreover, there are other types of methods that specifically treat MPWW such as activated sludge with reverse osmosis (Bohdziewicz & Sroka, 2005); activated sludge with coagulation (Sroka *et al.,* 2004); ultrafiltration with reverse osmosis (Bohdziewicz & Sroka, 2002); coagulation with reverse osmosis (Bohdziewicz & Sroka, 2003), aerobic treatment by using SBR (Ge *et al.,* 2013); and a combination of coagulation with ultrafiltration and reverse osmosis. Bohdziewicz & Sroka (2006) have found that some of these technologies have exhibited efficiency in the removal of pollutants from MPWW. However, the disadvantages which include toxic by-products, high cost as well as the adverse

effects on the environment have limited the application of these technologies. The complexity of each industrial activity is the main reason why the treatment of wastewater should be specifically tailored to each industry (Liu *et al.,* 2014).

The application of microalgae in the bioremediation of pollutants is known as the phycoremediation process. It has been known to be eco-friendly and cost effective in the removal of pollutants (Sriran & Seenivasan, 2012; Phang *et al.,* 2015) since the process only requires adequate sunlight and a few nutrients such as nitrogen and phosphorus to grow (Aslan & Kapdan, 2006). Moreover, microalgae also have the ability to assimilate pollutants under different environmental conditions as claimed by Dwivedi, (2012); Worku & Sahu, (2014). Additionally, growing algae in wastewater has more advantages over traditional algae farms; they not only assimilate nutrients, but also offer economic sustainability as MPWW is rich in nutrient loading and does not require the addition of synthetic nutrients to support algae growth (Lu *et al.,* 2015). Thus, coupling algae with wastewater such as MPWW can result in significant benefits not only in terms of production cost but also the environment through nutrient consumption and carbon dioxide fixation (Liu *et al.,* 2014). However, the current practices in microalgae technologies are either too expensive or unstable as most commercial cultivations of microalgae use synthetic chemicals for microalgae growth. Hence, replacing chemicals with wastewater may reduce the cost of production (Lu *et al.,* 2015).

Scenedesmus sp. is a type of green algae that widely exists in freshwater bodies. According to Li *et al.,* (2011b), *Scenedesmus sp.* has a good potential in exhibiting and removing nutrients such as nitrogen and phosphorus as well as lipid accumulation (Song *et al.,* 2014; Gupta *et al.,* 2016). Additionally, a study done by Park *et al.,* (2010) showed that *Scenedesmus sp.* can remove effluents that contain high amounts of ammonium and alkalinity due to its own characteristics for growth. Moreover, *Scenedesmus sp*. has the ability to survive in adverse and robust conditions of pH, temperature, light and salinity. It also has high growth rates under higher concentrations of bicarbonates and CO_2 gas (Tripathi *et al.,* 2015). These characteristics make *Scenedesmus sp.* a suitable candidate in treating MPWW as MPWW contains high nutrient concentration which can be essential for microalgae

growth as claimed by previous researchers. (Park *et al.*, 2010; Xin *et al.*, 2010; Alva *et al.*, 2013; Song *et al.*, 2014; Kim *et al.*, 2015; Lekshmi *et al.*, 2015; Gupta *et al.*, 2016; Michel *et al.*, 2016). The results have proven that Scenedesmus sp. is capable of removing pollutants successfully from different types of wastewater.

Thus, the aim of this research is to investigate the phycoremediation potential of *Scenedesmus sp.* in removing the nutrient pollutant load of MPWW. It includes a preliminary study to identify the survivability of *Scenedesmus sp.* under different environmental conditions that enhance growth and biomass production such as pH, temperature, light intensity and photoperiod. Furthermore, a laboratory experiment was conducted to assess the potential of *Scenedesmus sp.* for cultivation under natural weather conditions, its availability in treating wastewater, as well as its potential by-products. Treating MPWW is an economic and health necessity as claimed by Bustillo-Lecompte *et al.*, (2016).

1.2 Problem Statement

Wastewater produced from the meat processing industry should be discharged correctly and be free from harm pollutants. Failure to do so can cause major environmental hazards such as eutrophication due to excessive nutrients. This results in the deterioration of water quality which indirectly harms human health and aquatic life. Nevertheless, one of the meat processing factories located at Parit Raja Darat, Johor, currently throws and discharges highly polluted wastewater directly from the factory into the nearest rivers without any treatment prior to discharge. Such activities can cause adverse effects to small rivers and other nearby water bodies such as eutrophication, death of aquatic life and unpleasant smells resulting from the discharge. Typically, meat processing wastewater (MPWW) is known to be rich in nutrients such as nitrogen, phosphorus and carbon. These elements are essential for all forms of life. An innovative and sustainable method using sustainable materials and approaches is crucial in assessing this problem. Recently, the idea of applying phycoremediation technology using the microalgae *Scenedesmus sp.* in treating MPWW is promising. Mostly, microalgae has the

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